THE

Malayan Agricultural Journal.

Vol. XVI. FEBRUARY, 1928. No. 2.

The Bitter-Cucumber or Peria.

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The cucumber family *Cucurbitaceae* supplies several useful and highly-prized vegetables in Malaya. Among those commonly cultivated are the pumpkin or labu manis (*Cucurbita pepo* D.C.); gourd or labu (*Cucurbita maxima*, Duchesne); cucumber or timun (*Cucumis sativus*, Linn); wax-gourd or kundor (*Benincasa cerifera*. Savi.); loofa or kĕtola (*Luffa acutangula*, Roxb and *L. aegyptica*, Mill.); bottle-gourd or labu jantong (*Lagenaria vulgaris*, Ser.) and the snake-gourd or kĕtola ular (*Trichosanthes anguina*, Linn.) There are others not yet as well known as these esteemed locally by Chinese, Indians and Malays and among them is the bitter-cucumber or pĕria (*Momordica charantia*, Linn.) which forms the subject of this article.

In India and Ceylon this plant is known as karoli, karela, or kerola and in the West Indies as cerasee.

The species is widely distributed in the tropics and sub-tropics and often occurs as a troublesome weed, but in Malaya it is rarely met with in a truly wild state, although it is occasionally found in places where it has escaped from cultivation. The reason why it is not capable of spreading locally is no doubt due to the fact that its fruit is nearly always badly attacked and destroyed by the fruit-fly (*Chaetodacus cucurbitae*, Coq.) to which further reference will be made later.

All the striking varieties of the bitter-cucumber grown in the Peninsula appear to have had their origin under cultivation, probably in South China and India, from the one

species, judged only from observations on their vegetative, flowering and fruiting characters, which observations, however, have not yet been supplemented by lengthy breeding and cytological studies.

Although the plant is grown chiefly for its fruit, which is reputed to be very wholesome when cooked and eaten as a vegetable, yet other parts of it are said to be of value for medicinal purposes. According to Heyne all parts of the plant contain a non-glucosidal bitter principle and he describes several native methods of preparing bitter decoctions from the stem, leaves and fruit for use as tonics and for various internal ailments.

In 'The Useful Plants of India' Drury states that "the whole plant mixed with cinnamon, long-pepper, rice, and marothy oil (*Hydnocarpus inebrians*), is administered in the form of an ointment in psora, scabies, and other cutaneous diseases. The juice of the leaves mixed with warm water is reckoned anthelmintic. The whole plant pulverised is a good specific externally applied in leprosy and malignant ulcers".

The following translation of an old Malay proverb taken from the Journal of the Straits Branch of the Royal Asiatic Society, Vol. 1 p. 89:—aptly describes the plant's bitterness.

"You may plant the bitter-cucumber on a bed of sugar, manure it with honey, water it with treacle and train it over sugar-cane, but when cooked it will still be bitter." Moral: Natural habit cannot be eradicated.

Because of the bitter taste, few Europeans find the vegetable palatable, however, if the sliced fruit is steeped in salt and water for some time before being boiled in fresh water, it loses a good deal of its bitter flavour. When prepared in this manner the long tender varieties, although slightly bitter, are quite good-eating especially with curries.

Heyne (loc. cit.) gives the following directions for preparing the fruit for consumption.

"The fruits are sliced and boiled. The water is poured off and they are then stewed. Thus prepared they are considered very effective in purifying the blood. A more palatable way of cooking them is to slice them lengthwise, take

out the pulp and substitute dried fish, meat and spices and then wrap them in leaves of the culinary lemon grass plant (*Cymbopogon citratus* Staph.) and stew them."

If the fruit is sliced and thoroughly dried it can be kept for some months before being used.

Description of the Plant.

The following description is based mainly on notes made on living specimens grown in the Kuala Lumpur Plantation.

The plant is a slender climbing leafy herb with a life history extending over a period of about 3 months. The stem is 6 feet long or more and hairy especially near the apex. The leaves have an average length of 3—4 inches and width of 4—5 inches; they are alternate, hairy on the main veins, deeply 5—7 lobed, coarsely toothed with the apices of the lobes, sharply pointed. The petiole is 2—3 inches long and often slightly hairy. The tendrils are 6—9 inches long and usually simple, but sometimes 2—branched along half their length.

The wiry peduncle of both the male and female flowers is 3—4 inches long and bears a green, leaf-like entire and kidney-shaped bract which is usually situated at or below the middle of the stalk.

The flowers are solitary, axillary, unisexual, very rarely hermaphrodite, and yellow in colour.

The male flower is 1—1½ inches in diameter. It consists of five pale green ovate sepals ⅓ as long as the petals. The five petals are obovate, free to the base and ribbed. The three stamens are shortly stalked and attached to the sides of the mouth of the short tube where they form a short column. Two of the stamens are 2—celled and one 1—celled. The anther lobes are curved and contain smooth, elliptical, yellow pollen grains.

The female flower is, as a rule, slightly smaller than the male flower. It consists of 5 small lanceolate pointed persistent sepals, ¼ as long as the five petals. Each of the three short styles surmounting the ovary is divided near the apex so that there are as a rule six stigma lobes. At the base of the styles are three glands—2 large and one small.

The ovary is inferior about ½ inch long and composed of three carpels. The ovules which number 16—25 or more are arranged almost horizontally and borne in two lines on the margins of each of the three placentas which are attached to the wall of the ovary.

The fruit is a large berry, oblong-ellipsoidal in shape, tapering to both ends, with eight to ten thick longitudinal ridges between which are numerous smooth irregular tuberculate outgrowths. The unripe fruit is pale green and filled with soft white pulp. When ripe the fruit is orange-yellow in colour and bursts open at the apex into three segments exposing the seed.

The seed is large, flat, oblong 13 m.m. long by 7 m.m. wide; shortly pointed at each end and immersed in soft deep crimson juicy pulp. The hard outer seed-coat is corrugated along the margin of the seed and sculptured on the surface.

The above description of the plant may perhaps be usefully supplemented by a few notes concerning growth and development.

The seed as previously described, possesses a hard testa which enables it to retain its vitality for a long period under conditions not suitable for germination. When sown in the field, or nursery and well-watered, germination takes place rapidly and the young seedlings appear in 7 to 10 days. In germinating, the embryo as it swells, ruptures the testa laterally into two equal portions. The central part of the the embryo, the hypocotyl, has on the lower side of its base where it adjoins the root-zone, a peculiar flange like out-growth which serves to hook down the lower half of the testa until it is completely separated into two parts, thereby enabling the hypocotyl to withdraw readily the cotyledons as it lengthens upwards to form an arch above the surface of the soil and before it straightens itself out. In other respects the different phases of germination do not call for special comment.

The seed-leaves quickly become green on exposure and these, as the young stem lengthens, are followed by a pair of opposite, rounded, cordate and slightly 3 or 5 lobed foliage leaves. The next pair of leaves are usually almost opposite and deeply 5—7 lobed. After this, as growth proceeds, all the leaves are definitely alternate and each one, commencing commonly with the fifth, bears a long tendril in its axil.

Axillary branches quickly appear after the first few leaves have been formed so that the supports of the plant are soon covered with a dense mass of stems, tendrils and foliage.

The first flower, which is a male one, usually appears in the axil of the fifth foliage leaf and two or more male flowers follow in succession, one to each leaf, until a female flower occurs. The female flowers produced are relatively few in number so there is nearly always a preponderance of male flowers, and no difficulty is experienced in obtaining pollen for selfing the female flowers in breeding experiments.

The male flower opens early in the morning and the anthers burst and shed their pollen shortly after. By evening the petals close loosely and either the same evening, or next morning, the whole flower is abscissed at its base leaving the long peduncle and its bract attached to the plant where they remain.

The female flower, like the male flower, opens early and remains receptive for one day only. The following day the petals fall leaving the sepals, style and stigmas, all of which organs persist and can be readily seen even when the fruit is ripe.

The flower is insect-pollinated. In the Kuala Lumpur district where the observations were made, pollination was effected chiefly by small bees.

After fertilization the young fruit develops rapidly and ripens in about three weeks after the opening of the flower. To date all bagged female flowers from which pollen was excluded failed to set fruit.

Varieties.

From an economic standpoint the different varieties of the bitter-cucumber are of more interest and importance than the type described although this is grown, but not commonly, for its fruit and for medicinal purposes.

At the Malayan show held in Kuala Lumpur in August 1927 fruit of six distinct and striking varieties were exhibited by Chinese market gardeners. Seed of these were collected and grown, and subsequent investigations showed that they represented fairly completely the chief market forms. The

largest varieties were said to have been imported originally from China where no doubt most of them have been grown over a long period of years.

All the fruit exposed for sale in the local markets is full-sized, but unripe, and almost white due to etiolation in the paper covers with which it has to be protected from the ravages by the fruit-fly. Besides affording the necessary protection from damage, the paper cover is said to lead to the production of tender and less bitter fruit.

In trial cultures it was found necessary to enclose completely the female flower in paper bags immediately after pollination in order to be certain of obtaining sound fruit. Any fruit not so protected was quickly attacked by the fly and damaged.

The leaves, flowers and ovaries of the long and large fruited varieties are bigger than those of the original type and would probably show distinct differences in the number and character of the chromosomes in their cells similar to those found in other cultivated plants where giant and other peculiar forms occur.

Unlike the long hot-house variety of the cucumber (*Cucumis sativus, var.*) where fertile seed is produced only in the apical portion, the varieties of the bitter-cucumber produce viable seed along the greater portion of the inside length of their fruit. Measurements of young ovaries at flowering time showed that they varied from ½ inch in the original type to 1—1¼ inches in the large varieties. The former contained ovules as a rule along a length of 3/10 inch whilst the latter had ovules covering 5/10 inch or more. In a ripe fruit 3 inches long of the type described perfect seed occurred along two inches of the inner wall whilst in a 14 inch fruit of one of the varieties (No. 5) they were found covering a length of no less than 10 inches.

The characters and average dimensions of ripe fruit and seed of the species (No. 1) and six varieties of it which were collected locally and grown are as follows:—

1. *The type.* Fruit oblong—ellipsoidal, narrowed to both ends, 3 inches long and 1¼ inch in diameter at centre. Main ridges 8—10, muricate or tuberculate between. Flesh up to ½ inch thick, Seeds 15—25 each 13 x 7 m.m.

2. *A small variety.* Fruit resembles that of No. 1 except that it is larger and rounder 4—5 inches long, 1½ inches wide at centre. Flesh ¼ inch thick. Seeds 20—40 rather larger than those of No. 1.

3. *A giant variety.* Fruit oblong cylindrical in shape with a very broad base but tapering to apex. Length 10—12 inches width at base 2¾ inches, at centre 2¼ inches, Main ridges large and much interrupted, tuberculate excrescences large, flesh up to ½ inch thick. Seeds 25—40 each 14 x 8 m.m.

4. *A medium-sized and favourite market variety.* Fruit oblong-cylindrical 9—12 inches long 1½ inches in diameter, tapering to both ends. Flesh rather more than ¼ inch thick. Seeds 25—50 each 14 x 8 m.m.

5. *A giant variety.* Fruit cylindrical tapering to both ends, 14—15 inches long 2¼ inches in diameter, Main ridges very thick. Flesh 2/5 inch thick. Seeds 25—40 each 15 x 8.5 m.m.

6. *A giant variety.* Fruit cylindrical with a rather broad base which is much smaller than that of No. 3. 12—14 inches long 2¼ inches wide. Main ridges not continuous, tubercules very numerous. Flesh ½ inch thick. Seeds 25—40 each 14 x 8 m.m.

7. *A medium-sized variety.* Fruit resembles that of No. 4 but smaller 6—8 inches long and 2 inches wide. Flesh ¼ inch thick. Seeds 20—45 each 13 x 7 m.m.

The remarkable increase which has taken place in the size of the fruit is of particular interest, for the fruit of the wild plant is only about 3 inches long, whereas that of some of the varieties reaches a length of 12 to 15 inches. Again the edible portion— the wall of the fruit—is much thicker in most of the cultivated forms whilst the seeds are larger and more numerous.

Although the bitter-cucumber is of somewhat minor economic importance at the present time, yet its reputed good qualities both as a food and for medicinal purposes, might be worth investigating.

Summary.

(1) The bitter-cucumber or pĕria (*Momordica charantia*, Linn.) and certain varieties of it are described.

(2) It is shown that the fruit is much esteemed as a vegetable in Malaya by Chinese, Indians and Malays, who ascribe to it, as well as to other parts of the plant, certain medicinal properties.

(3) It is suggested that the nature and possible value of the active bitter principle found in the plant might be worth studying.

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Explanation of Plates.

Plate I. Dried specimens of seedlings (I) and (II) and a branch (3) of the bitter-cucumber (*Momordica charantia*, Linn.) showing vegetative and flowering characters.

Plate II. Bitter-cucumber varieties as grown in experimental cultures.

Plate III. (1) Fruit of four varieties of the bitter-cucumber compared with the fruit of the original type No. 1, at left. The fruit of Nos. 3 and 5 as depicted is below average size, but shows its chief characters.

Plate III. (2) Fruit of the bitter-cucumber as exposed for sale in the local markets.

Received for publication 4th February, 1928.

Dried Specimens of Seedlings (1) and (2) and a branch (3) of the bitter-cucumber (Momordica charantia, Linn.) Showing vegetative and flowering characters.

PLATE I.

Bitter-cucumber varieties as grown in experimental cultures.

PLATE II.

Fig I. Fruit of four varieties of the bitter-cucumber compared with fruit of original type (1) at right. The fruit of numbers (3) and (5) as depicted is below average size but shows its chief characters.

Fig II. Fruit of the bitter-cucumber as exposed for sale in the local market.

A Preliminary Note on Phytophthora spp. Found in Malaya.

Aldworth Thompson.

The fungus *Phytophthora* is one of the most important parasitic fungi in phytopathological work all over the world. Its ravages are responsible for considerable losses to cultivators of annual crops, particularly to the potato crop. In Malaya, however, it has up to now only attracted attention on two hosts namely rubber (*Hevea brasiliensis* Mull) and to a lesser extent, sireh (*Piper Betle* L.) That this is so is largely due to the position occupied by rubber as the major agricultural industry of the country.

The occurrence of *Phytophthora* spp., on other hosts, has been reported from India, Ceylon, the Netherland East Indies and other Eastern countries. These hosts include coconut palms, cacao, areca palms, castor oil, *Colocasia*, tobacco, papaya, nutmeg, *Dendrobium* sp. and *Odontadenia* sp.

The host list from Europe and America is a large one and many species of *Phytophthora* are involved, but in the Eastern tropics the principal species concerned are *P. palmivora* Butler (which is synonomous with *P. faberi* Maubl.) and *P. meadii* McRae, *P. arecae* Coleman, *P. nicotianae* Breda de Haan, *P. colocasiae* Rac. and *P. parasitica* Dastur.

Phytophthora on rubber, first came under observation in Ceylon, as the cause of Claret-Coloured Canker and Pod-rot. Petch (1) in 1905 stated that these diseases were due to a fungus similar to that which causes the decay of Cacao pods in Ceylon, and, by inoculations he showed (2) in 1910 that the cacao fungus—*Phytophthora faberi* Maubl.—was capable of causing Canker and pod disease of rubber. He concluded from this that the original cause of these rubber diseases was *P. faberi* Maubl. but did not report that he had isolated *P. faberi* Maubl. originally from rubber, and proved its connection with the diseases according to the laws formulated by Koch.

P. faberi Maubl. has since been isolated from rubber pods by Gadd in Ceylon (3) and shown by him to differ from the cacao strain. Gadd separates *P. faberi* Maubl. in Ceylon

into two groups, the cacao group and the rubber group, according to observed morphological differences when grown in pure culture on Maize meal agar, and from the production of oospores when a member of the cacao group is grown, in mixed culture, with a member of the rubber group. As a result of cross inoculation experiments he states "Though the *Phytophthora* from cacao will readily infect *Hevea* pods under laboratory conditions, it would appear from general observations that a similar infection does not readily occur in nature." He also states that "the results of the inoculations on cacao, rubber, breadfruit, and papaw fruits indicate that these fruits are most readily attacked by the strains which were originally isolated from them, and that the majority of the other strains will infect these fruits under suitable conditions. In general, *Hevea* pods are more readily attacked by all strains than are the other fruits used in these experiments. Though the various strains readily attack *Hevea* pods, the *Phytophthora* from *Hevea* does not attack other fruits with equal readiness."

As a result of Gadd's work the position with regard to *P. faberi* Maubl. in Ceylon, has been rendered much clearer. He has shown that the pod disease of *Hevea* is due to a biological variety of *P. faberi* Maubl. With regard to Patch Canker however more work is required to find which strain of *P. faberi* Maubl. is responsible for this disease, as the investigations of Petch (loc cit) and Rutgers (4) only prove that the cacao strain can attack rubber bark.

In Malaya, the writer has obtained three fungi from Patch Canker of *Hevea*. One of these is a *Pythium* sp. formerly reported (5) as a *Phytophthora*, and the other two are respectively *P. faberi* Maubl. and a *Phytophthora* sp. which differs from *P. faberi* Maubl. in culture. The *Pythium* sp. and *P. faberi* Maubl. were obtained from Perak and the other *Phytophthora* from Selangor. The *P. faberi* Maubl. from Perak is morphologically indistinguishable from *P. faberi* Maubl. isolated by the writer from Black Stripe disease of *Hevea* in Malacca, and from its behaviour in mixed culture it is considered to be identical.

The *Phytophthora* from Patch Canker in Selangor grows well on Maize meal and Potato-dextrose agar, producing a fair quantity of aerial mycelium. Sporangia and chlamydospores are not produced for some time and then only spar-

ingly when compared with the abundant numbers produced by the Black Stripe fungus *P. faberi* Maubl. A marked difference in the growth of the two fungi is seen when they are grown on slices of papaya fruit which have been sterilised in a hot air chamber at 120°C. for half an hour. The Black Stripe fungus *P. faberi* Maubl. produces only a slight growth of aerial mycelium, but abundant sporangia and chlamydospores on the surface of the fruit. The Patch Canker fungus produces large quantities of aerial mycelium, but no sporangia or chlamydospores until fourteen days and then only a few are found. Comparative measurements of sporangia on Potato-dextrose agar show a marked difference in length and in the ratio of length to width, and the behaviour in mixed culture with various strains of *P. faberi* Maubl. is also different. The Black Stripe fungus forms oospores when grown with *P. palmivora* Butler, and, when grown by Ashby at the Imperial Bureau of Mycology, Kew, also formed oospores in mixed culture with a cacao strain from Trinidad. No oospores have been obtained, so far, when the Trinidad cacao strain and the Black Stripe strain have been mixed in Malaya. Mixed cultures with the Selangor Patch Canker fungus have produced no oospores when grown at Kew or in Malaya.

Inoculation experiments with the Malayan fungi isolated from Patch Canker of *Hevea* have been carried out. Those for the *Pythium* sp. have been described (5) in 1925. In 1927 the writer inoculated a number of rubber trees with the Black Stripe fungus and with the Selangor Patch Canker fungus. Results to date indicate that the fungus isolated from Patch Canker causes more serious patches than the Black Stripe fungus, while the Black Stripe fungus causes more serious Black Stripe disease than the Patch Canker fungus. Details of these will be published later.

Mr. S. F. Ashby of the Imperial Bureau of Mycology, Kew, considers that the Selangor Patch Canker fungus has much in common with *P. nicotianae* Breda da Haan. Rutgers (loc cit) has shown, however, that *P. nicotianae* Breda de Haan does not attack *Hevea*. He states "The chief results of these infection experiments is the certainty that the *Phytophthora* spp. which occur on other cultivated plants, particularly on tobacco, are harmless to rubber, except those occurring on cacao and nutmeg."

The writer has received from Kew two cultures of *P. nicotianae* Breda de Haan one obtained originally from Rutgers and one from Chicago. It is intended to compare the Selangor Patch Canker fungus with these.

Dastur (6) in 1915 obtained a *Phytophthora* from Black Stripe disease of *Hevea* (known as "Black Thread") in Burma. Sharples et al (7) consider that this fungus is clearly related to the Malayan Black Stripe fungus in which case it is different to the Selangor Patch Canker fungus. The writer hopes to obtain a culture of the Burma fungus, for comparative study, to decide this point. It is also possible that the fungus isolated from Black Stripe in Malaya and described (7), is either a different strain to the fungus obtained recently by the writer from Black Stripe in Malacca, or is slightly changed in form through constant association with bark of *Hevea*. It would be interesting to obtain numerous cultures of the Black Stripe fungus from different localities in Malaya and to compare them. It is quite possible that several different strains would be obtained.

Pod-rot and Secondary leaf-fall.

Up to the end of 1927, there was no record of Pod-rot of *Hevea* in Malaya with which *Phytophthora* was associated.

The disease is known in Ceylon and S. India, and is followed by a leaf fall which often assumes serious proportions. In Ceylon the causal fungus is *P. faberi* Maubl. and in South India it is due to *P. meadii* Mc. Rae. The latter fungus differs from *P. faberi* Maubl. principally in the formation of oospores in pure culture. In 1925 some of the pods, on a tree in Selangor which had been inoculated on the bark with the Patch Canker *Pythium* sp., developed a rot and fell before ripening. They were found to be attacked by the *Pythium* sp., which was obtained in culture. A careful watch, for similar trouble, was kept until recently, but no further cases have been found.

Towards the end of 1927 the writer found some diseased rubber pods, which had been produced late in the season, and from these obtained a *Phytophthora* which produces abundant oospores, of the 'parasitica' type, in pure culture. In some respects this fungus differs from *P. meadii* Mc. Rae as described by Mc Rae (8) in 1918.

Cultures of the fungus were sent to Mr. Ashby of the Imperial Bureau of Mycology, who reports that only oospores were produced in culture and no other reproductive bodies. Sporangia are produced in fair quantity in Malaya, and a few chlamydospores are also found. Measurements of seventy sporangia from Potato-dextrose agar give a mean length of 42.98 microns and a mean width of 28.0 microns; measurements of one hundred oospores gives a mean diameter of 19.4 microns. Oospores measured at Kew show a larger mean diameter, the average of fifty being 21.1 microns on Potato-dextrose agar and 22.6 microns on Maize agar.

Little work has been done with this fungus so far but a few preliminary inoculations on young rubber leaves did not produce any serious disease. A culture of the fungus has been sent to the Rubber Research Institute of Malaya to facilitate progress, in case any outbreak of secondary leaf fall due to this fungus should appear this year.

Phytophthora sp. on Sireh (Piper Betle L.)

As reported by the writer (9) in 1926, a *Phytophthora* sp. was isolated from a wilt of sireh in Pahang. This fungus was grown in culture in Malaya for some time, but produced no oospores. The writer went on leave to Europe in 1926, and took home a culture of this fungus. When grown at the Imperial Bureau of Mycology on Maize, Oat, and Bean Agar, it produced oospores in fair numbers. These were of the 'parasitica' type, but were much larger than those of *P. parasitica* Dastur, ranging from 19—31.5 microns (mean=25.6 microns) on Oat agar, and from 19.5—26.3 microns (mean=22.1 microns) on Maize agar, and from 19.7 to 27.4 microns (mean=23.1 microns) on Bean agar. These figures were determined by Mr. S. F. Ashby who suggests that the fungus has much in common with *P. colocasiae* Rac. On returning to Malaya the fungus was maintained in culture, but did not produce oospores. A type culture of *P. colocasiae* Rac. obtained from Kew, has been grown on the same media. This fungus produced oospores at Kew and continues to produce them in quantity in Malaya. The sireh fungus began to die out soon after its return from England, and efforts to invigorate it by passing it through susceptible hosts were unsuccessful. A vigorously growing culture has now been obtained from Kew, and it is hoped to carry the investigation further. The disease is also reported

from India by Dastur (10) and in India it appears to be seriously injurious to *Piper Betle* L. Dastur states that the fungus in India produces oospores similar to *P. parasitica* Dastur. From his description of the Indian fungus it appears to have some points in common with the Malayan species, particularly in the germination of the sporangia. In Malaya the fungus when first obtained produced sporangia many of which produced zoospores on germination, but after a few months in culture, zoospores were not produced and the sporangia germinated by germ tubes. When grown in England however, active zoospores were easily produced.

Climatic influence appears to play a large part in the growth of *Phytophthora* spp. and renders the identification of a *Phytophthora* a matter of considerable difficulty, in the tropics. Sharples et al. (loc. cit.) state "The very careful work of Rosenbaum (11) suggests that on similar media under similar conditions considerable reliance can be placed on measurements, best expressed as biometric constants. It appears to be essential, however, that comparisons should be made in the same place on exactly similar media and for prolonged periods. The necessity for such work is shown by the fact (communicated to the writers by Mr. Dastur) that *P. meadii* grown at Pusa produces sporangia approximating in size and shape to those of the Burma fungus and varying markedly from those produced in S. India. Even more care is necessary when effecting separations on the presence or absence of spore forms; in addition to the well known facts of the occurence of non-sporing strains and of intermittent sporing there are climatic influences to be considered e.g. *P. meadii* at Pusa produces resting spores while the Malayan fungus grown there does not even produce sporangia."

Climatic or some other influence apears to modify the form of *Phytophthora* spp. considerably when certain species are grown in a country different from that of their origin. The behaviour of the Malayan *Phytophthorae* in India and England renders it difficult positively to identify a species. Leonian (12) has studied the saltation phenomenon in *Phytophthora* and also the effect of different hosts on the form of sporangia produced by any one species (13). He concludes that "no new species or varieties have been produced by saltation and that while the rate and type of colony growth, the shape and size of sporangia, and the presence or

absence of oogonia are greatly influenced by saltation, these characteristics are too unstable and reversible to be given primary specific importance."

It would appear that the best method of obtaining a key to identify Malayan species of *Phytophthora* is to grow as many type species of *Phytophthora*, as possible, in Malaya, under similar conditions and for a prolonged period, to record their behaviour under these conditions and to use the results as a basis for classification of existing Malayan species and of those which may be isolated in the future.

Received for publication 10th February, 1928.

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Notes on Sclerotium Rolfsii Sacc. in Malaya.

Aldworth Thompson.

Sclerotium Rolfsii Sacc. is a fungus which possesses no known conidial stage and which persists by means of hard, small, round, brownish bodies known as sclerotia. These bodies are formed by the intertwining of hyphae to form a compact parenchymatous mass, white at first, later becoming brown.

The host plants of *Sclerotium Rolfsii* Sacc. are numerous; it attacks ground nut (*Arachis hypogea* L.) coffee, (*Coffee spp.*), sweet potato, (*Ipomea batatas* L. Poir) tomato, (*Lycopersicum esculentum* Mill.) potato, (*Solanum tuberosum* L.), pepper (*Capsicum annuum* L.), sugar cane (*Saccharum officinarum* L.), and rice, (*Oryza sativa* L.) and many others. In Malaya it has been specially noticed on Jerusalem Artichoke (*Helianthus tuberosus* L.), sireh (*Piper Betle* L.), *Crotalaria usaramoensis* E. G. Baker, *Tephrosia candida* W.C., tapioca (*Manihot utilissima* Pohl), ground orchid (*Spathoglottis plicata* Blume), and soya bean (*Glycine hispida* Maxim), rice (*Oryza sativa* L.) and *Caladium spp.*

The fungus is almost always associated with rotting of the parts of plants in contact with the ground, especially the tubers, roots, and collar and, given the right conditions of temperature and humidity, can spread rapidly and do considerable damage. It has been reported to attack the tapped bark of *Hevea* in Sumatra (1), but the damage done is insignificant.

The Fungus on Jerusalem Artichokes.

Artichokes suffer severely from attacks of *Sclerotium Rolfsii* Sacc. Once a plant in a bed becomes infected, it is almost a certainty that the remainder of the plants will soon be attacked. When the soil has become infected with sclerotia, further crops of artichokes on that soil will be liable to attack, for many years, if some system of soil sterilisation is not adopted. The sclerotia of *S. Rolfsii* Sacc. are very resistant to drought, and, in the tropics, retain their powers of germination for an indefinite period. Laboratory experiments show that the sclerotia can germinate even when kept for ten months in a dessicator over Calcium chloride.

Symptoms.—The first indication of the presence of *S. Rolfsii* Sacc. in a bed of artichokes is the sudden wilting of a number of the plants. Examination of the stem at ground level reveals a strong growth of white mycelium, often in strands, closely applied to the tissues of the stem and extending over the neighbouring soil. This mycelium becomes attached to the stem by means of holdfasts, and kills the epidermal cells. After this the hyphae enter the tissue and the plant dies. The mycelium then extends to the roots and tubers, and the latter decay. According to Higgins (2) the death of the cells is due to the toxic action of oxalic acid secreted by the hyphae. If the attack is well advanced a number of white, or brown seed like bodies will be found on the mycelium or lying scattered about the soil near the affected plant. These bodies are the sclerotia of the fungus, which on germinating reproduce the disease if a susceptible host is planted in the infected soil.

When the disease appears in a plot for the first time it may not become evident until the plants are full-grown and the tubers ready for lifting. Subsequent crops on the same soil however, rarely grow to any size before being attacked.

In 1924 some Jerusalem Artichokes growing in the Government Plantation, Kuala Lumpur became infected with the fungus. About half the plants were attacked and it was hoped to save the remainder by careful lifting and replanting the tubers. The tubers were therefore removed and washed in water to remove the soil and replanted in fresh soil. Only a few produced new leaves and shortly after the fungus again reappeared and all the tubers rotted. The soil in the bed was well dug over several times, exposed to the sun, and left unplanted for 6 months. At the end of that period one half of the bed was given a liberal dressing of lime, and, a month later, artichokes were planted both in the limed and unlimed soil. In about a fortnight the places where the tubers had been planted were easily noticed owing to a strong growth of mycelium and sclerotia of *S. Rolfsii* Sacc. on the surface of the soil over each tuber. No essential difference in the amount of infection was observed in the soil dressed with lime.

On another occasion a plot of half an acre of artichokes was attacked when the tubers were ready for lifting. The tubers from plants apparently free from the disease were

removed and carefully washed and dried and put in bags. In a short time the fungus appeared on the tubers and the majority were rotted.

Another batch of tubers were washed and dipped in corrosive sublimate (1 : 1000) for half an hour, rinsed in water and dried. These remained free from the disease and produced healthy plants when grown.

Cultural Studies.—Pure cultures of the fungus were easily established on Maize-Meal Agar by planting sclerotia which had been previously immersed in corrosive sublimate (1 : 1000) for two minutes and then washed for two minutes in sterile water. Coarse white mycelium is produced when the sclerotia germinate; this occurs about 24 hours after the culture is put up. In four days white tufts of intertwining mycelium appear in the culture and by seven days these develop into white, shining sclerotia which remain white for a short period and then gradually darken to a brown colour. They remain attached to the mycelium for a long time, but as the culture medium dries up they become free. Frequently the sclerotia become united either in pairs or in a mass which forms an uneven crust on the surface of the media. When this occurs the sclerotia forming the crust lose their round shape and become flattened and irregular in outline.

Slices of fresh artichokes were washed in corrosive sublimate (1 : 1000) and sterile water and placed in sterile petri dishes. These were inoculated with sclerotia from a culture and kept at room temperature. Growth proceeded rapidly and in three weeks a crust of sclerotia had formed at the edges of the petri-dish and had lifted the petri dish cover about quarter of an inch.

Sclerotia vary in size and shape according to the culture medium used. If the growth is poor only a few small spherical sclerotia are produced. Where the growth is vigorous sclerotia are larger, being separate and spherical, or occuring in clusters forming a stroma-like crust. From 40—100 sclerotia may be produced in cultures obtained from a single sclerotium.

Measurements were made of sclerotia grown on five different media. Fifty representative spherical sclerotia

were chosen from each medium and the diameter ascertained. The figures were:—

	Mean of 50
Potato blocks	- 2.8mm.
Maize meal agar	- 3.1mm.
Meat extract agar	- 1.3mm.
Green Pea agar	- 1.3mm.
French Bean agar	- 2.8mm.

Mycelium.—Copious mycelium is produced when the fungus is grown in culture on Potato blocks, Maize-meal agar, and French bean agar. As indicated by the size of sclerotia it does not favour Meat extract or Green Pea agar, on which media only a small quantity of mycelium is produced. On boiled rice, no sclerotia were developed.

The mycelium is white, septate, and the larger hyphae contain many vacuoles. The hyphae in culture tubes run more or less parallel with each other along the length of the tube on each side of the point of inoculation, ending in a plug of mycelium at the upper end of the slant. The diameter of the hyphae varies from 2.1—10.5 microns on Maize meal agar. When placed in water for examination under the microscope they appear to be united in strands. Clamp connections are to be found in the larger hyphae.

Inoculation Experiments.—A dozen Artichoke tubers were procured, and planted in pots in soil sterilised by heat. The tubers were immersed in corrosive sublimate (1:1000) for twenty minutes and washed in sterile water before planting. The depth of planting was one inch below the surface in the case of six tubers, and two inches in the case of the other six. Young sclerotia from cultures of *S. Rolfsii* Sacc were then scattered over the soil. All the tubers germinated after a month but only two survived. The remainder, in all the pots, were attacked soon after the appearance of the leaves and died. The two survivors would probably have been attacked later on, but the experiment was stopped. *S. Rolfsii* Sacc. was re-isolated from the stem tissues. The disease was left to run its course in three pots, and in two of these fresh artichoke tubers were planted after three months had elapsed. The tubers did not produce leaves and when examined were found to have rotted. Numerous sclerotia were produced on the surface of the soil over each tuber. The third pot was kept indoors in a dry place for 18 months

after which it was placed in the open for two months until weeds appeared. Artichokes planted in this pot were not attacked by the disease.

A half dozen tubers were sterilised as before in corrosive sublimate, washed in sterile water and each one placed in a sterile covered jar. Two tubers were inoculated by placing a sclerotium from a young culture on the surface. Each sclerotium had been soaked for one minute in corrosive sublimate and well washed in sterile water. The sclerotia germinated and caused the tubers to rot.

A few dessicated sclerotia from a year old culture were placed in water until they became swollen. These failed to germinate when placed on the tubers. They were then removed and placed in Maize agar. Growth occurred, and young sclerotia were produced which, on inoculation, caused the tubers to rot.

Some of the same batch of sclerotia were placed for one minute in corrosive sublimate and then soaked for an hour in sterile water. These failed to grow either when inoculated into tubers or when cultured on Maize agar.

No spore stage was encountered either in culture or in the field.

Control Measures.—To control this disease economically is a matter of extreme difficulty if the area is at all large. On very small plots, such as are found in school gardens in Malaya, it may be possible to remove the infected soil carefully and replace it with fresh soil, preferably burnt earth. Before removing the soil the dead stems, leaves and tubers should be lifted and well burnt on the soil, in small heaps. As will be seen from the photograph on Plate I the soil is infected to a depth of at least four inches, and to be on the safe side six inches of soil should be removed. The soil can be scraped into a trench and covered with burnt earth, so that the infectious material is about a foot underground. It would be advisable not to grow artichokes or other tuber-rooting crop in the plot for some months.

To control the disease on a larger area some system of chemical treatment is required. Trials with lime have not been satisfactory in this country, but a report (3) from the Phillipines states that damage from a disease of Tomato and Pepper caused by *S. Rolfsii* Sacc. can be reduced in pot plants

by the addition of lime to the soil at the rate of three or more per cent, by weight, while Sulphur, Copper stearate, Corona 640, and Uspulun also gave promising results.

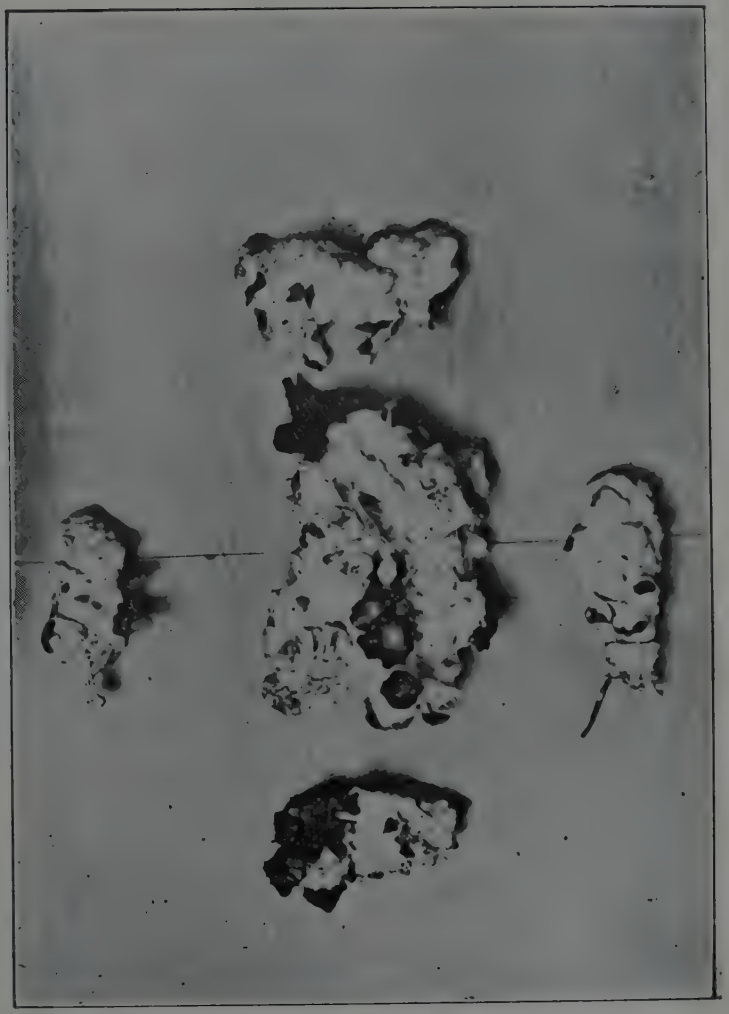
Sclerotium Rolfsii Sacc on Rice.

In 1927 some rice grains were being tested for germination. Some of the germinated grains developed a covering of coarse white mycelium, which on being cultured proved to be *S. Rolfsii* Sacc. In culture, the fungus resembles *S. Rolfsii* Sacc. from artichokes in the mycelial characters; the sclerotia, however, develop more quickly and are smaller, ranging from 0.8 mm. to 1.8 mm. on Glucose--peptone agar, from 0.9 mm. to 2.0 mm. on Potato-dextrose agar, and from 1.0 to 2.0 mm. on Maize agar. Sclerotial tufts appear in culture on the second day and on the sixth day the majority of the sclerotia are fully developed and brown in colour. On boiled rice only mycelium was produced at first, sclerotia not developing for five weeks.

Inoculations.—Eight Roux potato tubes were arranged having water in the bulb and a plug of cotton wool in the tube over the water, and in contact with it. The tubes were lightly plugged and then sterilised in the autoclave, and, when cold six rice grains were put into each tube on the top of the moist cotton wool. All the rice grains germinated and produced healthy green plants, with strong roots. Four of the tubes were inoculated by placing mycelium from a culture on boiled rice into the centre of the growing plants. Two of the remaining tubes were inoculated by scattering sclerotia from a culture on Potato dextrose, over the cotton wool round the plant roots, and two tubes were kept as a control.

After three days the plants in the tubes inoculated with mycelium had become infected. The mycelium was growing up the culms of all the plants which were discoloured brown for half an inch from the base upwards. Two days later the plants turned yellow and in four days more sclerotia were forming on the wool and all over each plant. Eleven days after the experiment was begun all the plants had died. The plants in the tubes inoculated with sclerotia only, were not attacked nor did the sclerotia germinate. These plants and the controls grew vigorously until the experiment was stopped.

Artichoke plant killed by *S. Rolfsii* Sacc. showing white mycelium and young white sclerotia in the soil.



Artichoke tubers covered with the white mycelium of *S. Rolfsii* Sacc.

(2) Young rice plants, growing in a pot in which the soil was kept wet, were inoculated by placing mycelium of the fungus from boiled rice, and mycelium and sclerotia from Potato-dextrose Agar at the base of each plant. The pot was covered with a belljar to produce a humid atmosphere. The fungus grew on the soil for a couple of days and then attacked the outer leaf sheaths of the plants and killed them. The attack then ceased, the fungus disappeared and the plants continued to grow well. Pieces of a young green rice plant were then scattered over the soil between the plants. The fungus again made its appearance and grew strongly on the decaying pieces and from these spread to the growing plants but only attacked the outer leaf sheaths and then disappeared. The same procedure was tried once more but the fungus only appeared in places and did no more damage.

The belljar was then removed, the soil moisture was reduced and an artichoke tuber was planted in the pot with the rice. The artichoke grew well and was not attacked. A fresh supply of sclerotia and mycelium was placed in contact with the artichoke stem, at the base, but the result was negative, even though the stem was lightly wounded later. Further inoculations with the fungus on artichokes in pots also proved negative, indicating that the fungus on rice is of a different strain to that on artichokes. A negative result was also obtained when soya bean and sireh plants were inoculated with the fungus. The experiment was repeated and on this occasion pieces of rice plants were placed in the soil and round each plant. The fungus appeared on the soil but the plants were not attacked.

Rice in Malaya is comparatively free from disease, and to date there is no record of any serious loss caused by sclerotial diseases. In India a fungus *Sclerotium oryzae* Catt. is responsible for the loss of a certain amount of the crop. This fungus was first described by Cattaneo (4) in 1879, when he reported it as the cause of serious damage to rice in Novara and Lombardy.

A sclerotial disease is also reported from the Phillipines (5) where it causes a considerable amount of damage in the rice fields. Out of 126 varieties, 105 were infected. The disease is serious only in areas which are very weedy and where the soil is very moist. In fairly dry soil, free from weeds, no infection was noticed. It is also recorded that flooded rice areas are free from the disease. From the descrip-

tion of the fungus it is possible that it is a strain of *S. Rolfsii* Sacc. but its identity with the Malayan strain on rice is uncertain. The Phillipine fungus was shown to be parasitic to Soya bean seedlings, while the Malayan fungus failed to attack this host. The plants used in Malaya however were about half grown and further inoculations are necessary to decide the point. In many other respects the fungus is similar to the Phillipine strain and it is intended to look for cases of disease in dry padi areas in Malaya. Two other sclerotial fungi have been recorded on rice, namely *Sclerotium glumale* Ces. which attacks rice in Borneo, and a species *S. irregulare* described by Miyake as causing some loss in Japan. These two fungi are distinguished from *S. oryzae* Catt. by the size of the sclerotia. The writer has been unable to obtain the literature dealing with *S. glumale* Ces. or *S. irregulare* Miyake, but since the Malayan fungus was identified by the Imperial Bureau of Mycology as *S. Rolfsii* Sacc., it is recorded under that name.

Towards the end of 1927, the writer isolated two other sclerotial fungi from a rice disease in Perak. One of these forms small brown or black sclerotia somewhat larger than the black sclerotia, of *S. oryzae* Catt. while the other forms large sclerotia, many of which remain permanently white on the outside, sometimes having a brown internal structure. It is hoped to continue work with these fungi when time permits.

Sclerotium Rolfsii Sacc. on Sireh (Piper Betle L.)

In November 1927, specimens of *Piper Betle* L. were received from Temerloh in Pahang. The base of the stem was covered with white mycelium and a few small sclerotia were present. Cultures were easily obtained and the fungus proved to be *S. Rolfsii* Sacc. Mycelial characters in culture were the same as those of the fungus from artichoke and rice. Sclerotial Measurements were:—

Maize Agar	1.0 mm — 2.0 mm	(m = 1.4 mm)
Potato-dextrose Agar	0.8 mm — 6.0 mm	(m = 1.8 mm)
Boiled rice	nil	

The disease is first noticed when infected plants wilt. This occurs suddenly and happens soon after infection. On examination, the stem at the base is seen to be covered with small white sclerotia, which are sometimes formed on the outside of the epidermis before any noticeable growth of myce-

lium is seen. Usually only the first internode from the roots upwards is affected, the stem being hollow at this point due to rot of the internal tissues. After the production of sclerotia, the stem collapses at this node, and the remainder of the stem withers away. The roots were not directly attacked by the fungus.

Inoculations.—(1) Three sireh plants, growing in pots, were inoculated by placing a small portion of mycelium and some sclerotia on a small lump of soil. This was then placed in contact with the stem, without wounding, a quarter of an inch below the surface of the soil. In four days the plants wilted suddenly and small white sclerotia were being produced all over the first internode of each plant. The fungus was recovered in culture from these sclerotia.

(2) Two artichoke plants in a pot were inoculated by placing a little mycelium and a few sclerotia in contact with the stem at soil level. The following day mycelium was observed growing on the stem and two days later the lower leaves began to wilt. Nine days after the inoculation the plants died. The fungus was reisolated from the tissues of the plant and from sclerotia produced in abundance on the stem. The disease travelled up the stems for about two inches before the plants wilted entirely, and then travelled down until it reached the tubers underground. These were examined fifteen days after the plants had died, and were just being attacked. Two days later the tubers were covered with mycelium and sclerotia were being produced. Finally the tubers rotted and the top 2—3 inches of soil in the pot was full of sclerotia and mycelium.

(3) A sireh plant was inoculated with *S. Rolfsii* Sacc. obtained originally from artichokes. The plant died a week later and the fungus was recovered from the tissues.

As already stated, inoculations on sireh with the fungus from rice were unsuccessful, but rice plants in tubes were quickly attacked by the fungus from sireh and killed. Rice plants in tubes were then inoculated with the fungus from artichokes, but although mycelium grew on some of the germinating grains, there was no development of sclerotia on the young plants and the fungus was not observed to attack the green shoots.

Sclerotium Rolfsii Sacc. on Caladium sp.

Caladium plants are also liable to be attacked by this fungus. The tubers and bases of the leaves are affected, and sclerotia are produced in much the same manner as in the case of artichokes. The cultural characters of the fungus are similar to those already mentioned. Sclerotia measure from 0.8mm to 2.0mm on maize agar, from 1—3mm on Potato-dextrose Agar but are not produced when the fungus is grown on boiled rice. Inoculations on *Caladium* spp. with the fungus were successful when fresh sub-cultures were used, but not if the cultures were over one month old.

Artichokes and Sireh plants, were successfully inoculated with this strain, but all inoculations on rice plants were unsuccessful. On the other hand when *Caladium* plants were inoculated with the strains from artichokes, sireh, and rice success was obtained on each occasion, but the strain from artichokes caused death more rapidly than did the other two.

The result of these inoculation experiments may be rendered clearer by the following table where + = successful inoculation and — = unsuccessful inoculation.

Strain from	Effect on Host.			
	Artichoke	Caladium.	Rice.	Sireh.
Artichoke -	+	+	—	+
Caladium -	+	+	—	+
Rice -	—	+	+	—
Sireh -	+	+	+	+

As will be seen from the above the strains from artichoke and *Caladium* appear to be identical as far as the experiments go, but the sireh strain differs in its effect on rice, and the rice strain differs in its effect on artichokes and sireh.

The experiments however should be carried further, over a larger host range, before any definite conclusions can be drawn. It is hoped to do this when time permits, and also to study more closely the morphology and physiology of the

fungus in culture. No definite spore form has as yet been demonstrated in *S. Rolfsii* Sacc. as far as the writer is aware. It would be interesting to grow these strains in mixed culture to see if any changes occur, although, since the fungus is not comparable to a *Phycomycete* such as *Phytophthora faberi* Maubl, in which oospores are produced in mixed culture, it is unlikely that a spore form would appear.

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Received for publication 27th February, 1928.

Publications of the Department of Agriculture.

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C = Cancelled